specifically, as well as the original claims. No new matter has been introduced by this amendment.

## I. Rejection Under 35 U.S.C. § 112, First Paragraph

Claims 21-23, 25-27, and 31 have been rejected as containing subject matter which is not described in the specification.

While Applicants disagree with the Office's position and believe that the subject matter of the claims is sufficiently described, solely in an effort to further prosecution of this application and not in acquiescence to the rejection, the claims have been amended to delete the phrase "at a pH of about 6.8 to produce insulin, under conditions where no crystals are formed." Therefore, Applicants request that the rejection be withdrawn.

## II. Rejections Under 35 U.S.C. § 103(a)

A. The Examiner has maintained the rejection of claim 21 under 35 U.S.C. § 103 as allegedly obvious over the Markussen references, in view of Goedell et al. and the Grau references. The Examiner argues that Markussen et al. ('212) discloses insulin precursors that are single peptide chains converted to human insulin by derivatization and treatment with trypsin. The Examiner admits the Markussen references do not specifically teach the preparation of mono-Arg-insulin or the use of trypsin as a cleavage agent for generation of mono-Arg-insulin. The Examiner cites Goeddel et al. as teaching the production of recombinant fusion proteins of insulin

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precursors with another protein and cleaving them. The Examiner also states that Goeddel et al. teaches production in *E. coli.* The Examiner cites Grau ('684) as using trypsin and carboxypeptidase B simultaneously to produce mature insulin from proinsulin. Grau ('332) allegedly teaches that treatment of proinsulin with trypsin alone gives intermediates with an arginine at B31. This derivative allegedly is stable to further tryptic degradation. According to the Examiner, enzymes having both tryptic and carboxypeptidase B activity are required to produce insulin.

The Examiner states that it would have been obvious to one of ordinary skill in the art at the time the invention was made to use the intermediate with an arginine at B31 as suggested by Markussen et al. ('212) for the production of mono-Arg-insulin because mono-Arg-insulin is exceptionally stable to further tryptic degradation.

The Office has not established a *prima facie* case of obviousness because the applied references would have failed to:

- teach or suggest the starting materials used in the claimed process;
- teach or suggest the simultaneous addition of trypsin and carboxypeptidase as presently claimed.

Contrary to the assertion of the Office, there is no suggestion to make substitutions from the preferred embodiment of Markussen, nor any reasonable expectation of success in doing so. Markussen discusses a precursor represented by a generic formula B(1-29)-$(X_n$-$Y)_m$-A(1-21), which includes a very large number of species. In fact, a simple calculation shows that this formula includes millions of possible compounds which may be used as the starting material to produce insulin.

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The Federal Circuit has addressed this issue in *In re Baird*, 15 F.3d 380 (Fed. Cir. 1994). In *Baird*, the court stated that a disclosure of millions of compounds does not render obvious a claim to three compounds. *Id.* at 1552. The Court held that there was nothing in the prior art suggesting that one of ordinary skill should select the claimed compound from the numerous compounds contained within the broad genus. Applicants refer to the Patent and Trademark Office's new "Genus-Species Guidelines" (62 F.R. 6217), which became effective on February 11, 1997, changing the Office policy to incorporate the holding in *Baird.* Thus, not complying with the law set forth in *Baird*, the Office has not provided reasons why one would have selected the miniproinsulin as the starting material to produce insulin, especially given the large number of possible compounds included within Markussen's generic formula. Stability of mono-Arg-insulin does not render Applicant's miniproinsulin obvious, since mono-Arg-insulin does not fit within the generic formula of Markussen as suggested by the Examiner.

The Examiner states that Grau ('684) teaches the simultaneous addition of trypsin and carboxypeptidase. (*See* column 5, lines 49-59.) The Examiner has rejected Applicants' arguments from the prior response distinguishing Grau from the present invention. The Examiner has argued that the argument contrasting the natural porcine proinsulin of Grau with the miniproinsulin of the present invention is not persuasive. The Office, here, alleges that Applicants cannot attack the references individually.

As discussed above, this is a misapplication of the law. It certainly is relevant that Grau does not remedy the deficiencies of Markussen by disclosing the claimed

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mono-Arg-insulin. Further, Grau describes cleavage of a different compound and there is no assurance and no reasonable expectation of success that this would work with the miniproinsulin of the present invention. The deficiencies in this reference must be considered when evaluating the nonobviousness of the present invention, as no other reference compensates for the deficiency.

Applicants respectfully request the withdrawal of this rejection.

B. The Examiner has maintained the rejection of claim 25 under 35 U.S.C. § 103(a) as allegedly obvious over the Markussen references, Goeddel et al., the Grau references, and Mai et al. The Examiner states that Markussen, Goeddel et al., and Grau do not specifically teach the bridging member Met-Ile-Glu-Gly-Arg of step (A) in the claim. According to the Examiner, Mai et al. teach that it would have been well known in the art to use common cleavage sites in fusion proteins. This reference teaches that cyanogen bromide cleaves after the amino acid methionine and that Xa cleaves after the tetrapeptide Ile-Glu-Gly-Arg. The Examiner states that both Markussen and Goeddel et al. suggest making fusion proteins, which can be cleaved.

In addition to the shortcomings addressed above, claim 25 is not obvious over the prior art as it has a novel and nonobvious bridging member. Here the Office admits that the bridging member is not taught in the prior art; however, indicating in so many words that it would have been obvious to try the combination. The Office fails to show that the prior art suggests this particular member, only pointing to general suggestions regarding fusion proteins. "Obvious to try" is not the state of the law. One skilled in the

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art must have had a reasonable expectation of success in making the proposed combination. M.P.E.P. § 2143. The Office has, thus, not established the requisite *prima facie* case of obviousness.

Applicants request the withdrawal of this rejection.

C. The Examiner has maintained the rejection of claims 22 and 23 under 35 U.S.C. § 103(a) as allegedly obvious over the Markussen references, either in view of Goeddel et al. or the Grau references. The Examiner believes it would have been obvious to use both trypsin and carboxypeptidase B to convert the miniproinsulin of Markussen first to mono-Arg-insulin, and then to insulin.

In addition to the arguments presented above regarding claim 21 above, Applicants believe that the Office has not shown the formation of mono-Arg-insulin as an intermediate. Merely because Grau ('332) teaches that this compound resists further tryptic degradation, the Office has not satisfied its burden. No evidence has been presented regarding the use of this compound *as an intermediate*. Applicants respectfully request the withdrawal of this rejection.

D. The Examiner has maintained the rejection of claims 26-27 and 31 under 35 U.S.C. § 103(a) as being unpatentable over the Markussen references, in view of either Goeddel et al., Mai et al., or the Grau references. Again, the Examiner states that it would have been obvious to use both trypsin and carboxypeptidase B to convert the miniproinsulin of Markussen et al. first to mono-Arg-insulin, and then to insulin.

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Further, according to the Examiner, Grau teaches that mono-Arg-insulin can be formed by trypsin cleavage and that this form is resistant to further tryptic degradation and Grau ('684) teaches that the combination of trypsin and carboxypeptidase B together can convert proinsulin to insulin. Further, as the Examiner has stated above, Markussen et al. and Goeddel et al. allegedly suggest making fusion proteins, and Mai et al. teaches a cleavable sequence such as used in the claimed invention.

The Examiner contends that one of ordinary skill in the art would have been motivated to select the species of Grau, which allegedly fits into the starting material genus of Markussen, and to carry out the presently claimed method. The Examiner, however, fails to explain how the prior art of record would have taught combining such a species as the starting material in the Markussen process, and modifying that process to arrive at the claimed process.

Applicants submit that the prior art of record would not have suggested using the species of Grau in the Markussen process as alleged by the Examiner. In fact, Applicants wish to clarify the record concerning apparent misperceptions of the Examiner concerning miniproinsulin and mono-Arg-insulin. In the present claims, miniproinsulin is the first starting material and mono-Arg-insulin is the final product in some claims (Claims 21 and 25) or an intermediate in other claims (Claims 22-23, 26-27, and 31).

The Examiner asserts that mono-Arg-insulin is a species of miniproinsulin and is encompassed within the broad genus disclosed by Markussen. Mono-Arg-insulin is not a species of miniproinsulin as alleged by the Examiner. Mini-proinsulin comprises a

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single amino acid chain comprising the B-, C and A-chain of insulin. Mono-Arg-insulin comprises two amino acid chains connected via disulfide linkages. Moreover, it does not appear that the mono-Arg-insulin of Grau fits within the generic formula of the starting precursor of Markussen (see, e.g., the formula at the bottom of column 2 in the '212 patent). Also, Markussen does not appear to disclose a generic formula that would encompass mono-Arg-insulin at any stage of the process.

Markussen '212 and Markussen (EPO) discuss methods for producing "insulin precursors." (Markussen '212, at col. 2, lines 33-39, for example.) The "insulin precursors" of Markussen differ from the recited mono-Arg-insulin and insulin in that they have not been properly converted into the two chain form of insulin. For example, the "natural" single chain precursor to insulin is the single chain B-C-A polypeptide, where the C chain is removed by proteolytic cleavage to convert the polypeptide into a two chain insulin molecule. (See Figure 29-10 in the excerpt of Zubay's "Biochemistry" submitted with the Amendment filed Aug. 2, 1996.) If the Examiner needs another copy of this article, please contact the undersigned.

The required "in vitro conversion" to arrive at a process producing mature human insulin, as discussed in the Markussen documents, refers only to a process involving L-threonine esters. (Markussen '212, at col. 5, lines 3-11; and Examples 14-18 at col. 18, line 25, through col. 19, line 33.) Markussen's use of this method supports a conclusion that those skilled in the art would not have expected trypsin to cleave at the C-terminus of a bridging Arg residue in the single chain "precursor" (the Arg of the recited formula B(1-30)-Arg-A(1-21)) to ultimately generate the two chain, mature form of insulin. Why

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else would one go through the additional steps involved in the L-threonine ester and not discuss or even mention other more direct methods such as cleavage with trypsin? Accordingly, Applicants' invention of methods wherein trypsin can be used in cleaving the single chain "precursor" into the final insulin or mono-Arg-insulin is not taught or suggested by the Markussen documents. In fact the Markussen documents teach different methods that suggest against cleavage with trypsin.

Furthermore, Applicants have argued that Thim et al. indicate that trypsin cannot cleave a miniproinsulin with a single Arg bridge between the B and A chains. (Amendment filed November 6, 1995, at page 11.) Markussen '212 discusses such cleavage of a fusion protein at the N terminus of the B chain rather than cleavage of the single chain B(1-29)-$X_n$-Y-A(1-21) "insulin precursor" to generate the mature, two chain insulin. (See Markussen '212, at col 3, lines 36-41.) For example, "[t]he insulin precursors may be expressed with additional protein proceeding the insulin precursor." (Markussen '212, at col. 3, lines 52-53; see also col. 4, lines 19-24 discussing N-terminal "superfluous amino acid sequence.") This enzymatic cleavage results in a two chain insulin or mono-Arg-insulin product according to the claimed invention.

Next, in addition to failing to establish that the art of record would have suggested modifying the methods of Markussen to arrive at the claimed methods for the reasons discussed above, the Examiner has also failed to suggest using the specifically claimed miniproinsulin (B(1-30)-Arg-A(1-21)) in any method, let alone Applicants' claimed method. Markussen discusses a precursor represented by a generic formula B(1-29)-$(X_n$-Y$)_m$-A(1-21), which includes a very large number of

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species. In fact, a simple calculation shows this formula includes about $3.6 \times 10^{43}$, possible compounds, which may be used as the starting material to produce insulin.

Moreover, as noted above, the Examiner has provided no reasons why one would have selected the miniproinsulin as the starting material to produce insulin, especially given the large number of possible compounds included within Markussen's generic formula. Stability of mono-Arg-insulin does not render Applicants' miniproinsulin obvious, since mono-Arg-insulin does not fit within the generic formula of Markussen as suggested by the Examiner.

The Examiner contends that Grau ('684) teaches the simultaneous addition of trypsin and carboxypeptidase B at Col. 5, lines 57-59. Applicants previously pointed out that Grau ('684) dealt with processes for obtaining insulin precursors rather than processes for obtaining insulin from mono-Arg-insulin. The Examiner disputes this assertion, contending that column 5, line 57-59, discusses using the two enzymes simultaneously to produce insulin.

Applicants point out that the method disclosed in Grau ('684) is different from the method of the present invention since Grau ('684) describes a **natural porcine proinsulin** isolated from pancreas while the instant invention describes a mini-proinsulin of the formula B(1-30)-Arg-A(1-21).

Since it is known that trypsin cleaves at the carboxy terminus of the basic amino acids arginine and lysine, in the case of porcine proinsulin, trypsin can cleave theoretically at 6 different sites in the molecule as indicated in the enclosed figure (2 sites in the B-chain, 3 sites in the C-chain and 1 site between C- and A- chain).

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Applicants inform the undersigned that, because the cutting rates (kinetics) of the possible cuts depend on the amino acid environment at each site, the actual cutting rates at the six different sites are different. For example, Applicants assert that the cutting rates at Arg-(B22) and Lys-(B29) must be relatively low compared to at least the rate of the cutting site at Arg-(C35) since the process described by Grau ('684) yields as main product insulin with intact B-chain. (The Arg-(C1) is cut off by carboxypeptidase B.)

The mini-proinsulin of the process of the present invention is part of a fusion protein. The fusion part is connected via a bridging member (MIEGR) to the mini-proinsulin having a C-chain with just one amino acid (Arg). Cutting off the rest of the bridging member (IEGR) - after removal of the fusion part by CNBr - is provided by trypsin, which cuts also at other sites in the mini-proinsulin part of the fusion protein (Arg-(B22), Lys-(B29) and Arg-(C1)). However, cutting off the mini-proinsulin from the bridging member occurs at a site which has no equivalent in the porcine insulin described by Grau ('684). In addition, Applicants assert that the cutting rate of trypsin at this new site could not have been predicted in view of Grau ('684). Therefore, Applicants assert that the present invention would not have been obvious, since it was surprising that the relative cutting rate of trypsin at the Arg of the bridging member is high enough compared to the cutting rates at Arg-(B22) and Lys-(B29) to give a good yield of human insulin. If the cutting rates were for example, roughly identical, one could not expect a good yield of intact human insulin, let alone a situation in which the

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cutting rates at Arg-(B22) and Lys-(B29) were higher than the cutting rate at the Arg of the bridging member.

For all of these reasons, Applicants respectfully request the withdrawal of this rejection.

## CONCLUSION

In view of the foregoing amendments and remarks, Applicants respectfully request the reconsideration and reexamination of this application and the timely allowance of the pending claims.

If there are any additional fees due under 37 C.F.R. §§ 1.16 or 1.17 which are not enclosed herewith, including any fees required for an extension of time under 37 C.F.R. § 1.136, please charge such fees to our Deposit Account No. 06-0916.

Respectfully submitted,

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